

WHAT IS CLAIMED IS:

1. An isolated polypeptide having protease activity for a specific protease cleavage site, wherein the protease activity is specific for a substrate peptide having an amino acid sequence comprising:



wherein R_1 and R_3 are independently any D or L isomer amino acid, R_2 is Ala or Gly, and wherein the specific protease cleavage site is between Asp and R_2 .

2. The isolated polypeptide of claim 1 wherein the substrate peptide is at least eight amino acids in length.

3. An isolated IL-1 β pro polypeptide having IL-1 β pro activity and encoded by a DNA sequence comprising:

a. a DNA insert selected from the group consisting of the nucleotide sequences in Seq. I.D. No. 1 beginning at nucleotide 1 and extending to nucleotide 1232, beginning at nucleotide 374 and extending to nucleotide 1232, and beginning at nucleotide 374 and extending to a nucleotide from about 851 to about 962,

b. DNA sequences which detectably hybridize to one or more of the foregoing DNA inserts and which code or express a polypeptide displaying biological activity to proteolytically cleave human precursor IL-1 β polypeptide at a cleavage site between the Asp 116 and Ala 117 residues; and

c. DNA sequences which, due to degeneracy of the genetic code, encode a mammalian IL-1 β pro polypeptide encoded by any of the foregoing DNA inserts and sequences.

4. The isolated polypeptide of claim 3 wherein the protein sequence is selected from the group consisting of amino acid 1 to amino acid 404, amino acid 51 to amino acid 404, amino acid 120 to an amino acid beginning at position 278 and extending to amino acid 315, and amino acid 120 to amino acid 404 of Seq. I.D. No. 2.

5. An isolated DNA sequence encoding a mammalian IL-1 β pro enzyme.

6. The isolated DNA sequence of claim 5 wherein the mammalian IL-1 β pro enzyme is a human IL-1 β pro enzyme.

7. The isolated DNA sequence of claim 5 wherein the DNA sequence comprises:

a. a DNA insert selected from the group consisting of the nucleotide sequences in Seq. I.D. No. 1 beginning at nucleotide 1 and extending to nucleotide 1232, beginning at nucleotide 374 and extending to nucleotide 1232, beginning at nucleotide 374 and extending to a nucleotide from about 851 to about 962;

b. DNA sequences which detectably hybridize to one or more of the foregoing DNA inserts and which code or express a polypeptide displaying biological activity to proteolytically cleave human precursor IL-1 β polypeptide at a cleavage site between the Asp 116 and Ala 117 residues; and

c. DNA sequences which, due to degeneracy of the genetic code, encode a mammalian IL-1 β pro polypeptide encoded by any of the foregoing DNA inserts and sequences.

8. A recombinant expression vector comprising a DNA sequence according to claim 5.

9. A recombinant expression vector comprising a DNA sequence according to claim 6.

10. A recombinant expression vector comprising a DNA sequence according to claim 7.

11. A process for preparing a mammalian IL-1 β pro enzyme or an analog or derivative thereof, comprising culturing a suitable host cell comprising a vector according to claim 8 under conditions promoting expression.

12. A process for preparing a mammalian IL-1 β pro enzyme or an analog or derivative thereof, comprising culturing a suitable host cell comprising a vector according to claim 9 under conditions promoting expression.

13. A process for preparing a mammalian IL-1 β pro enzyme or an analog or derivative thereof, comprising culturing a suitable host cell comprising a vector according to claim 10 under conditions promoting expression.

14. A method for improving wound healing at a wound site comprising administering a pharmaceutical composition to the wound site comprising the isolated polypeptide of claim 1 in a suitable pharmaceutical carrier.

15. A method for treating arthritis comprising administering a pharmaceutical composition

comprising the isolated polypeptide of claim 1 in a suitable pharmaceutical carrier.

16. A method for treating an autoimmune disease in a susceptible individual comprising administering a pharmaceutical composition comprising the isolated polypeptide of claim 1 in a suitable pharmaceutical carrier.

17. The method of claim 16 wherein the autoimmune disease is selected from the group consisting of Insulin-dependent diabetes melitus, Graves' disease, Hashimotos disease and a lupus disease.

18. A method for reducing the detrimental side effects of radiation treatment comprising administering a pharmaceutical composition comprising the isolated polypeptide of claim 1 in a suitable pharmaceutical carrier.

19. An antisense oligonucleotide comprising a sequence of at least 15 nucleotides complementary to a sequence of IL-1 β pro cDNA, wherein said antisense oligonucleotide inhibits translation of IL-1 β pro mRNA.

20. A compound comprising an amino acid sequence of from 1 to about 5 amino acid residues having an N-terminal blocking group and a C-terminal Asp residue connected to an electronegative leaving group, wherein said amino acid sequence substantially corresponds to at least a portion of the sequence Ala-Tyr-Val-His-Asp, residues 112 to 116 of Seq. I.D. No. 3.

112, 114, 2

21. The compound according to claim 20 having the formula:

Sub 22

Sub 3
Q₂
Contd

Q₂ is 0 to 4 amino acids such that Q₂-Asp substantially corresponds to at least a portion of the sequence Ala-Tyr-Val-His-Asp, residues 112 to 116 of Seq. I.D. No. 3; and
Q₁ is an electronegative leaving group:

29. The composition according to claim 28 wherein Z is C₁-C₆ alkyl, benzyl, acetyl, C₁-C₆ alkoxy carbonyl, benzyloxy carbonyl or C₁-C₆ alkoxy carbonyl, benzyloxy carbonyl or C₁-C₆ alkyl carbonyl.

30. The composition according to claim 28 wherein Z is t-butoxycarbonyl, acetyl or benzyloxy carbonyl.

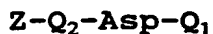
31. The composition according to claim 28 wherein Q₁ is an aldehyde, a diazomethyl ketone or a halomethyl ketone.

32. The composition according to claim 28 wherein Q₁ is fluoromethyl ketone.

33. The composition according to claim 28 wherein Q₂ is 1 amino acid residue.

34. The composition according to claim 28 wherein Q₂ is His, Phe, Pro or Tyr.

35. A method of inhibiting IL-1 β protease activity in a mammal in need of such treatment comprising administering to said mammal an effective inhibitory amount of a compound of the formula:



where Z is an N-terminal protecting group;

Q₂ is 0 to 4 amino acids such that the sequence Q₂-Asp substantially corresponds to at least a portion of the sequence Ala-Tyr-Val-His-Asp, residues 112 to 116 of Seq. I.D. No. 3; and

Q₁ is an electronegative leaving group.

36. The method according to claim 35 wherein Z is C₁-C₆ alkyl, benzyl, acetyl, C₁-C₆ alkoxy carbonyl, benzyloxy carbonyl or C₁-C₆ alkyl carbonyl.

37. The method according to claim 35 wherein Z is t-butoxy carbonyl, acetyl or benzyloxy carbonyl.

38. The method according to claim 35 wherein Q₁ is an aldehyde, a diazomethyl ketone or a halomethyl ketone.

39. The method according to claim 35 wherein Q₂ is 1 amino acid residue.

40. The method according to claim 35 wherein Q₂ is His, Phe, Pro or Tyr.

41. The method according to claim 35 wherein Q₁ is an aldehyde and inhibiting is reversibly inhibiting.

42. The method according to claim 35 wherein Q₁ is fluoromethyl ketone and inhibiting is irreversibly inhibiting.

43. A method of treating inflammation or preventing and treating an autoimmune disease in a mammal in need of such treatment comprising administering to said mammal an effective amount of a compound of the formula:

Sub 4
a



where Z is an N-terminal protecting group;

Q_2 is 0 to 4 amino acids such that the sequence Q_2-Asp substantially corresponds to at least a portion of the sequence Ala-Tyr-Val-His-Asp, residues 112 to 116 of Seq. I.D. No. 3; and

Q_1 is an electronegative leaving group:

44. The method according to claim 43 wherein Z is C_1-C_6 alkyl, benzyl, acetyl, C_1-C_6 alkyl carbonyl.

45. The method according to claim 43 wherein Z is t-butoxycarbonyl, acetyl or benzyloxycarbonyl.

46. The method according to claim 43 wherein Q_1 is an aldehyde, a diazomethyl ketone or a halomethyl ketone.

47. The method according to claim 43 wherein Q_2 is 1 amino acid residue.

48. The method according to claim 43 wherein Q_2 is His, Phe, Pro or Tyr.

49. A compound selected from the group consisting of Boc-Asp- CF_2F , Boc-His-Asp- CH_2F , Boc-Phe-Asp- CH_2F , Boc-Pro-Asp- CH_2F , Boc-Tyr-Asp- CH_2F , Ac-His-Asp- CH_2F , Ac-Phe-Asp- CH_2F , Ac-Pro-Asp- CH_2F , Ac-Tyr-Asp- CH_2F , Cb_3 -His-Asp- CH_2F , Cb_3 -Phe-Asp- CH_2F , Cb_3 -Pro-Asp- CH_2F , and Cb_3 -Tyr-Asp- CH_2F wherein Boc is t-butoxycarbonyl, Ac is acetyl and Cb_3 is benzyloxycarbonyl.

Sub 4
contd